A Probability-Based Modeling Approach for Characterization of ADC Charge Variants Separated by icIEF that Leverages Bottom-Up Mass Spectrometry Datasets

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CASSS – CE Pharm 2021
<table>
<thead>
<tr>
<th>AGENDA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Introduction - rationale</strong></td>
</tr>
<tr>
<td>• Challenges of indirect and direct charge variant (CV) characterization</td>
</tr>
<tr>
<td>• Addressing characterization challenges with models of CV separations</td>
</tr>
<tr>
<td><strong>Comparison between empirical and modeled CV distributions</strong></td>
</tr>
<tr>
<td>• Uncharged and charged ADC drug-linker models</td>
</tr>
<tr>
<td>• Chemical modifications of mAb backbone and drug-linkers (DL)</td>
</tr>
<tr>
<td><strong>CV modeling applications</strong></td>
</tr>
<tr>
<td>• Conversion of CV models to <em>in silico</em> intact MS</td>
</tr>
<tr>
<td>• Using modeling to enhance understanding of CE-MS and CEX-MS data</td>
</tr>
</tbody>
</table>
Charge variant assay interpretation

- Acidic variants
- Basic variants

Sample absorbance vs. pI

main peak

-80°C control
What do we do with this observed difference?

-80C control
40C stress
Analytics to insights approach

- What is it?
  - What are we observing in the assay?
- Why did it happen?
  - What is driving the change?
    - Deamidation or other PTM
    - DL hydrolysis
- Should we care?
  - Where does the change occur?
  - What is the impact for patients?
- What should we do?
  - Tailor control strategy to presumed criticality of the attributes that are changing

### Peptide map

<table>
<thead>
<tr>
<th></th>
<th>-80C control</th>
<th>40C stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>deamidation</td>
<td>1.1%</td>
<td>1.4%</td>
</tr>
<tr>
<td>DL hydrolysis</td>
<td>~2%</td>
<td>~9%</td>
</tr>
</tbody>
</table>
Direct characterization of CVs by OFFGEL fractionation

<table>
<thead>
<tr>
<th>Size-based Characterization</th>
<th>MS-based Characterization</th>
<th>Functional Assay Characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>R CE-SDS</td>
<td>Subunit</td>
<td>Binding</td>
</tr>
<tr>
<td>SE-UPLC</td>
<td>PTM Analysis</td>
<td>Potency</td>
</tr>
<tr>
<td>NR CE-SDS</td>
<td>NR Mass</td>
<td></td>
</tr>
</tbody>
</table>

OFFGEL

- Voltage
- Low pH
- High pH

pH Gradient Strip

Sample
OFFGEL as an ADC charge variant isolation strategy is artifactual

- OFFGEL: Direct characterization, but not viable for all ADCs
  - Observed assay-induced artifactual hydrolysis of drug linkers over duration of separation

![Graph showing absorbance over time for different time pools (Nominal, OFFGEL 12 Hour Pool, OFFGEL 16 Hour Pool). The graphs indicate changes in absorbance over time for different charge states (A4, A3, A2, A1, M, B). The data shows that the percentage of acidic species (A4, A3) changes over time, with notable hydrolysis observed in the 12 and 16 hour pools.]
CV profiles are complex and charged DLs will increase complexity

- Understanding of charge variants (CVs) is essential for developing ADC process and product knowledge
- The coming challenge: charged drug-linkers
  - Partially-loaded ADC species separate on the basis of drug-load
  - Additional complexity makes it very difficult to indirectly characterize and understand what is causing CV differences
- For all biologics there is a need for a holistic strategy that does not rely on fractionation and direct characterization of CVs

Uncharged DL

Charged DL
Binominal distributions are used to model CV profiles

- PTM input provided by reduced peptide map
- Statistical modeling via binomial distribution

\[
0.12^2 + 2(0.12)(0.88) + 0.88^2
\]
Model (expected) CV separation is generated from known molecular properties and direct PTM quantitation

- What do we know based on PTM molecular properties
- Apply basic probabilities and charge shift multiplier to all PTMs in peptide map data

<table>
<thead>
<tr>
<th>PTM</th>
<th>Assay</th>
<th>Location</th>
<th>Net charge shift</th>
<th>Statistical model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deamidation</td>
<td>Peptide map</td>
<td>Protein backbone</td>
<td>-1</td>
<td>Simple binomial</td>
</tr>
<tr>
<td>Hydrolysis</td>
<td>Peptide map</td>
<td>Protein backbone</td>
<td>-1</td>
<td>Weighted simple binomial</td>
</tr>
<tr>
<td>- Deamidation</td>
<td>Peptide map, LCMS</td>
<td>Drug-linker</td>
<td></td>
<td>Simple binomial</td>
</tr>
<tr>
<td>- Glycation</td>
<td>LCMS</td>
<td>Protein backbone</td>
<td>0</td>
<td>Simple binomial</td>
</tr>
<tr>
<td>Clip</td>
<td>LCMS</td>
<td>Unknown</td>
<td></td>
<td>Simple binomial</td>
</tr>
<tr>
<td>Oxidation</td>
<td>Peptide map</td>
<td>Protein backbone</td>
<td>1</td>
<td>Simple binomial</td>
</tr>
<tr>
<td>Succinimide</td>
<td>Peptide map</td>
<td>Protein backbone</td>
<td>-1</td>
<td>Simple binomial</td>
</tr>
<tr>
<td>Glycation</td>
<td>LCMS</td>
<td>Protein backbone</td>
<td>-1</td>
<td>Simple binomial</td>
</tr>
</tbody>
</table>
Combined model output is charge sorted and compared to icIEF

- Binomial modeling parameters
  - PTMs
    - Deamidation, Succinimide, N-term cyclization, C-term Lys processing, Glycation
  - DL hydrolysis

Model: 40C stress (Uncharged DL)

icIEF profile

Convert to discrete bars and compare with model
Binomial modeling tracks well for control and stress samples.
PTM differences between control and stressed material underly the profile changes observed in the CV model.
The impact of stress induced increases in PTMs and DL hydrolysis on CV separations can be abstracted.
Additional level of detail such as composition of PTMs in particular peaks can be inferred from modeled data.
Charged DL model to icIEF shows good agreement for stressed material

-80C control* 40C stress

*Note sample prep differences resulted in higher DL hydrolysis in -80C control
DL hydrolysis is the primary driver for CV profile change and increase in acidic species

Model: -80C control (Charged DL)

Model: 40C stress (Charged DL)
The modeling approach provides granularity into changes in specific molecular populations

- Categorical view for broad understanding
- Model provides greater granularity with enumeration of species with combinations of modifications

D: drug-linker
d: deamidation
g: glycation
h: DL hydrolysis

- other 1.4%
- DDDDdd 1.2%
- DDDDgd 1.4%
- DDdhh 0.3%
- DGDhh 0.4%
- DDDGh 8.9%
- DDDDh 19.9%
- DDDDhh 57.2%
- DDDDDD 9.3%
In silico mass spectrum generation from PTM-based CV model

- Compositional characterization allows for extended modeling opportunities
  - icIEF $\rightarrow$ CV profile model $\rightarrow$ theoretical mass spectrum

Charged DL:

- D: drug-linker
- d: deamidation
- g: glycation
- h: DL hydrolysis

Theoretical mass spectrum based on statistical species distribution
Merging intact CV-MS approaches with PTM-based CV modelling

- The development of MS compatible charge variant separations such as CEX-MS\textsuperscript{1}, CE-MS\textsuperscript{2} and CIEF-MS\textsuperscript{3} enhances understanding of separated proteoforms.
- PTM-based CV modelling is an orthogonal approach that can be leveraged to add complimentary, site-specific PTM information.

Advantages of utilizing PTM-based CV modelling

• Addresses the knowledge gap that exists when CV separations are not amenable to direct characterization through fractionation
• Provides a means to rapidly infer identities of new and changing peaks in analytical assays in a rigorous and quantitative manner
• Can be leveraged to better understand if a CV change is impactful to patients
  • Is the change due to a PTM in a mAb CDR potentially impact binding/activity
A big thank you to SeaGen’s Analytical Biochemistry department, particularly…

- Mass Spectrometry Core Group
- Analytical Sciences
- Process Analytics
- Formulation Sciences
- B5 Conjugation Team